

Prevalence of Toscana and Sicilian Phlebovirus Antibodies in Classic Kaposi Sarcoma Case Patients and Control Subjects in Sicily

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To assess whether arthropod bites promote Kaposi sarcoma (KS), we determined the seroprevalence of Sicilian (SFSV) and Toscana (TOSV) phlebovirus antibodies in 30 patients with classic KS and 100 controls in Sicily. Nine (6.9%) subjects, all controls, were positive for SFSV, whereas 41 (31.5%) were positive for TOSV. Seroprevalence with immunoglobulin (Ig) M or IgG against either virus was significantly higher in controls (43% vs 13.3% in case patients; $P < .01$). Adjusted for age, IgG seroprevalence was significantly lower in KS patients compared to controls (adjusted odds ratio, 0.22; 95% confidence interval, .07–.72). Low phlebovirus seroprevalence in patients with KS may reflect incapacity to produce robust, persistent antibody responses, and suggests that arthropod bites do not promote KS.

Classic Kaposi sarcoma (cKS) is a multifocal, low-grade vascular neoplasia that occurs in elderly persons, causing significant morbidity but little direct mortality. The necessary but insufficient cause of cKS is the human herpesvirus 8, also called Kaposi sarcoma–associated herpesvirus (KSHV). KSHV is transmitted mainly during childhood via saliva from the mother and others in the community [1]. KSHV distribution is heterogeneous, with seroprevalence ranging from 20% to 80% in

sub-Saharan African adults; 10%–20% in Mediterranean countries; and 0%–5% in Northern Europe, North America, and most of Latin America and Asia [2]. This extreme geographical variability has led many investigators to hypothesize several potential environmental risk factors that may influence KSHV prevalence as well as cKS incidence. Ecological investigations have considered latitude, climate, soil characteristics, vegetation [3], birth in areas with endemic malaria, and residence in proximity to rivers [4]. Based on these latter findings, a potential role of bites from bloodsucking insects has been postulated to explain KSHV transmission or perhaps viral reactivation. Moreover, a significant reduction in KSHV seroprevalence was observed after the larvicidal campaign against *Anopheles* mosquitoes in Sardinia [5]. More specifically, KSHV transmission is not supposed to be directly promoted by insects as biological/mechanical vectors, but indirectly when adults infected with KSHV rub their own saliva on a child's bite spot to relieve itching and swelling [6]. Several species such as Culicinae mosquitoes (*Aedes vexans* and *Ochlerotatus caspius*), sand flies (*Phlebotomus* spp), and biting midges (*Culicoides* and *Leptoconops* spp) that elicit strong skin reactions may represent such "promoter" arthropods. It was recently observed that the incidence of cKS in Sardinia was significantly correlated with the prevalence of arthropods that cause highly irritating bites, nearly all of which were *Phlebotomus* spp [7]. In particular, *Phlebotomus* spp are well-known vector insects of sandfly viruses, including Toscana virus (TOSV) and Sicilian virus (SFSV).

To further examine the arthropod-promoter hypothesis, we investigated the seroprevalence of TOSV and SFSV, considered a proxy of exposure to the *Phlebotomus* spp biting activity, in cKS patients and controls living in Sicily.

METHODS

Research Participants and KSHV Serology. The present study was carried out using sera collected during the 2002–2006 population-based cKS case-control study [8], which ascertained cases of cKS and randomly sampled controls from the entire island of Sicily. Subjects with indeterminate KSHV serology [8] and KSHV-seropositive control subjects were excluded from the current study, whereas cKS patients ($n = 30$) and KSHV-seronegative controls ($n = 100$) were a random sample of each subgroup. As reported in detail [8], seronegative subjects were nonreactive against KSHV latency-associated nuclear antigen and lytic antigens by immunofluorescence assay (IFA) and against KSHV K8.1 and open reading frame 73 antigens

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by enzyme immunoassay (EIA). The study was approved by institutional review boards at the University of Palermo, Italy, and at the National Cancer Institute in the United States.

TOSV and SFSV Serology Methods

All sera were analyzed as 1 batch for the presence of immunoglobulin (Ig) G- and IgM-specific anti-TOSV by EIA with recombinant N protein (IgG/IgM TOSV detection kit; DIESSE), according to the manufacturer's instructions. Those samples showing a borderline value were further analyzed by IFA to detect anti-TOSV IgM and IgG according to a procedure described elsewhere [9]. SFSV antibody detection was carried out using a commercial indirect immunofluorescence test (SfV IgG/IgM mosaic I; Euroimmun), as indicated by the manufacturer.

Statistical Analysis

All the data were analyzed using the R statistical software package version 2.2.0 [10]. The significance level chosen for all analyses was .05, 2-tailed. Absolute and relative frequencies were calculated for qualitative variables whereas quantitative variables were summarized as median (interquartile range). Categorical variables were analyzed using the χ^2 test (Mantel-Haenszel), Fisher exact test, or χ^2 for trend, as indicated. Adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were also calculated. All variables found to have a statistically significant association ($P < .05$) with seropositivity for TOSV and SFSV IgM or TOSV and SFSV IgG, or both, were entered in multivariate logistic regression models in order to check for independence. In the multivariate analysis, age was included as a continuous variable.

RESULTS

The general characteristics and the serological status of the 130 subjects in the current study are shown in Table 1. Consistent with the population-based sampling, subjects resided in all 9 provinces of Sicily, with the largest proportions from the more urbanized provinces of Palermo (24.6%) and Catania (22.3%). Overall, 9 (6.9%) subjects, all of whom were controls, were positive for SFSV, including 1 (0.8%) positive for IgM and 8 (6.2%) for IgG. Forty-one (31.5%) participants were positive for TOSV: 14 (10.8%) for IgM and 31 (23.8%) for IgG. These included 4 controls with both IgM and IgG, 10 controls with only IgM, 23 controls with only IgG, and 4 cKS patients with only IgG. IgM and IgG seroprevalence against TOSV and SFSV was unrelated to geography, whether classified on a north-south gradient or as coastal versus internal provinces ($P \geq .26$; data not shown). Other factors are presented in Table 2. The seroprevalence of IgM was significantly lower with older age ($P < .01$) and in cKS patients ($P = .04$); in the logistic regression analysis, cKS status lost significance but not age

Table 1. Characteristics of the Study Population

	Controls n = 100	cKS patients n = 30	Total n = 130
Sex, n (%)			
Female	22 (22)	14 (46.7)	36 (27.7)
Male	78 (78)	16 (53.3)	94 (72.3)
Age in y, median (IQR)	73 (63.5–77)	77.5 (73–81)	73 (65–79)
Blood draw season, n (%)			
Summer	15 (15)	10 (33.3)	25 (19.2)
Fall/spring	66 (66)	13 (43.4)	79 (60.8)
Winter	19 (19)	7 (23.3)	26 (20)
SFSV, n (%)			
Positive subjects	9 (9)	0 (0)	9 (6.9)
IgM-positive subjects	1 (1)	0 (0)	1 (0.8)
IgG-positive subjects	8 (8)	0 (0)	8 (6.2)
TOSV, n (%)			
Positive subjects	37 (37)	4 (13.3)	41 (31.5)
IgM-positive subjects	14 (14)	0 (0)	14 (10.8)
IgG-positive subjects	27 (27)	4 (13.3)	31 (23.8)

Abbreviations: cKS, classic Kaposi sarcoma; Ig, immunoglobulin; IQR, interquartile range; SFSV, Sicilian virus; TOSV, Toscana virus.

(adjusted OR, 0.94; 95% CI, .89–.99; data not shown in table). Significantly lower seroprevalence of IgG was found in cKS patients compared to controls ($P = .04$) and, in contrast with IgM, in younger compared to older subjects ($P = .01$). Both cKS status and age retained their significance in multivariate analyses (adjusted OR, 0.22; 95% CI, .07–.72 and adjusted OR, 1.05; 95% CI, 1.001–1.1, respectively; data not shown in table). Finally, seroprevalence with IgM or IgG against either virus was significantly higher in controls than in cKS patients (43% vs 13.3%; $P < .01$).

DISCUSSION

Although more than 16 years have passed since the discovery of KSHV in 1994, the precise routes of virus transmission remain unclear. Among the hypothesized risk factors, an effect of the irritating bites of some species of bloodsucking arthropods has been proposed to explain, at least in part, the geographical variability of KSHV seroprevalence and cKS incidence [7, 11]. To examine this hypothesis, we used the prevalence of antibodies against TOSV and SFSV as a marker of the exposure to the bites of their own vectors. Contrary to the hypothesis, our small, exploratory study found that seroprevalence against 1 or both of these arboviruses was significantly lower in cKS patients than in matched controls.

Because our controls are a representative sample of older adults in Sicily [8], we can estimate that the seroprevalence in this population is approximately 37% for TOSV and 9% for SFSV. The TOSV seroprevalence that we found in Sicily is higher than that reported with identical or equivalent serologic methods in

Table 2. Factors Associated With IgM and IgG Seropositivity Against TOSV or SFSV Among 130 Subjects in Sicily

	IgM seropositive		IgG seropositive		IgM or IgG seropositive	
	No. (%)	P value	No. (%)	P value	No. (%)	P value
Sex						
Female	3/36 (8.3)	.42 ^c	8/36 (22.2)	.33 ^a	10/36 (27.8)	.22 ^a
Male	11/94 (11.7)		29/94 (30.9)		37/94 (39.4)	
Age in y						
≤64	7/31 (22.6)	<.01 ^b	5/31 (16.1)	.01 ^b	10/31 (32.3)	.27 ^b
65–79	7/71 (9.9)		19/71 (26.8)		24/71 (33.8)	
≥80	0/28 (0)		13/28 (46.4)		13/28 (46.4)	
Blood draw season						
Summer	1/25 (4)	.08 ^b	9/25 (36)	.82 ^b	9/25 (36)	.29 ^b
Fall/spring	8/79 (10.1)		18/79 (22.8)		25/79 (31.6)	
Winter	5/26 (19.2)		10/26 (38.5)		13/26 (50)	
Classic Kaposi sarcoma						
Control	14/100 (14)	.04 ^c	33/100 (33)	.04 ^a	43/100 (43)	<.01 ^a
Case	0/30 (0)		4/30 (13.3)		4/30 (13.3)	

Abbreviations: Ig, immunoglobulin; SFSV, Sicilian virus; TOSV, Toscana virus.

^a χ^2 .

^b χ^2 for trend.

^c Fisher exact test.

other Italian regions (3% in Torino and 16% in Umbria), in Spain (5%–26%), or in France (12%) [12], but lower than in Corfu (51.7%) and Cephalonia (39%) [13]. Part of the higher seroprevalence that we found compared with other areas may be related to the older median age in the present study, as has been well associated by other authors with phlebovirus seropositivity [14]. Our seroprevalence also may be high because most of the Sicilian population, and thus most of our subjects, live in coastal areas where the climate is mild throughout the year. Mild weather could allow the vectors to circulate for a longer time and, consequently, to increase the probability of infection by these phleboviruses.

Our analyses confirm that increasing age is a strong risk factor for phlebovirus IgG seroprevalence. The lower IgM seroprevalence that we found with older age could point to a protective effect by IgG against acute phlebovirus infection or to the habits of older people to spend their time indoors or far from rural outdoor environments where arthropod vectors such as *Phlebotomus* spp are found.

IgM was detected in one-third of the TOSV-positive sera, including 7 of the 12 positive sera collected from subjects < 65 years of age. This is remarkably high, considering that an IgM response usually converts to an IgG response a few weeks following an acute infection. This observation could suggest that the viruses/arthropods were circulating more, and resulting in more acute infections, during the years of our study (2000–2006) than in earlier years. Alternatively, persistent IgM seropositivity may be a consequence of reinfection. Longitudinal data would be needed to examine these and related hypotheses.

In our study, IgG seroprevalence against phleboviruses was lower in cKS patients than in KSHV seronegative controls, even after adjustment for age. This appears to contradict Ascoli et al [4, 7], who reported an association between the density of vector insects and cKS. These observations may be compatible if we consider that host immunity may affect both humoral responses and risk for cKS. People at highest risk for KS are severely immunocompromised patients, such as human immunodeficiency virus–infected individuals and transplant recipients, as well as the elderly. Recently we found that cKS was associated with deficiencies in cellular immunity, both in vitro against KSHV-specific antigens and in vivo against common microbial antigens [15, 16]. Given these considerations, the phlebovirus seronegativity in cKS cases may be a consequence of their incapacity to produce robust and persistent antibody responses, rather than to a reduced exposure to arthropod bites.

The major limitations of the present study are its small size, use of seropositivity to only 2 phleboviruses as a proxy of exposure to the arthropod bites during the subject's lifetime, heavy weighting to the elderly, and exclusion of KSHV-seropositive controls without KS.

In conclusion, contrary to the hypothesis, the prevalence of TOSV and SFSV antibodies in cKS patients was lower than in KSHV-seronegative control subjects. Future studies, including studies with younger and disease-free KSHV-seropositive subjects, are needed to clarify if these findings may be linked to immunological mechanisms or to a reduced exposure to arthropod bites.

Notes

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